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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

F. Abel Ponce de Leon et al.

Group Art Unit: 1655

Application Serial No. 09/341,105

Examiner: B. Sisson

Filed: September 7, 1999

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TECH CENTER 1600/2900

Title: Z-CHROMOSOMAL MARKERS DERIVED FROM CHICKEN (*GALLUS DOMESTICUS*) AND  
USE THEREOF IN CHROMOSOMAL MAPPING

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AMENDMENT AND REPLY

Hon. Commissioner of Patents  
Washington, D.C. 20231

Sir:

This Reply is responsive to the final Office Action dated March 9, 2002. Kindly enter  
the following amendment and remarks prior to further examination.

IN THE SPECIFICATION:

The paragraph beginning on line 19 of page 5 is amended as follows:

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***Fluorescent in situ hybridizations***

C<sup>1</sup> -- The Z-chromosome-specific DNA fragments were fluorescently labeled by PCR  
with biotin-l6-dUTP (3:1 ratio of dTTP:biotin-l6-dUTP) and passed through a Sephadex  
G-50 column to remove unincorporated nucleotides. The protocol described by Ponce de  
Leon (*Proc. Natl. Acad. Sci., USA* (in press) (1996)) was followed. Briefly, 200 nanograms  
of labeled Z-chromosome specific DNA was mixed with 6  $\mu$ g of chicken competitor DNA  
(average size 200-400 bp) and 5.8  $\mu$ g of salmon sperm DNA (average size 200-400 bp),  
precipitated and resuspended in 12  $\mu$ l of hybridization buffer consisting of 50% deionized  
formamide, 1X SSC and 100% dextran sulphate to achieve a final DNA concentration of 1  
 $\mu$ g/  $\mu$ l. The hybridization mix was denatured at 75°C for 5 minutes and reannealed at 37°C